repolarization and refractoriness. Mutations in SCN5A that pathologically increase late INa cause type 3 long QT syndrome (LQT3). INa dysfunction from mutated SCN5A can depend upon the splice variant background in which it is expressed, and also upon environmental factors such as acidosis. S1787N was reported previously as a LQT3-associated mutation and has also been observed in 1 of 295 healthy white controls. Here, we determined the in vitro biophysical phenotype of SCN5A-S1787N in an effort to further assess its possible pathogenicity. Methods and results: We engineered S1787N in the two most common alternatively spliced SCN5A isoforms, the major isoform lacking a glutamine at position 1077(Q1077del) and the minor isoform which contains Q1077, and expressed them in HEK-293 cells for voltage clamp study. After 24h transfection, S1787N in Q1077 background had WT-like INa including peak density, activation, inactivation and late INa in both pHi 7.4 and pHi 6.7. However, with S1787N in the Q1077del background, the percentage of INa late/peak was increased 2.1 fold compared to WT in pHi 7.4 (n= 7-9, $p<0.05$) and was increased 2.9 fold compared to WT in pHi 6.7 (n= 6-8, $p<0.03$). In adult rat ventricular myocytes infected with an adenoviral recombinant of S1787N/Q1077del, action potential duration was prolonged in normal pH.
Conclusion: An LQT3-like biophysical phenotype for S1787N is both SCN5A isoform and intracellular pH dependent. These findings provide further evidence that the splice variant and environmental factors affect the molecular phenotype with implications for the clinical phenotype and may provide insight into acidosis-induced arrhythmia mechanisms.

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Dominant-Negative Suppression of Sodium Channel Activity by a Brugada Syndrome Mutation Observed in Cardiomyocytes

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Brugada syndrome (BrS) is an inherited cardiac disease with an autosomal dominant pattern. Unfortunately, the molecular and cellular mechanisms leading to BrS are not yet completely understood. Recently, the L325R-*SCN5A* mutation has been proposed to cause BrS through a dominant negative effect. This usually occurs if the mutant channels interact with wild-type (WT) channels forming non-functional channel multimers. However, sodium channel α-subunits are not believed to oligomerize. Therefore, we tested whether the dominant-negative effect seen in some BrS mutations is due to interactions between sodium channel α-subunits. Here we found that when WT-*SCN5A* and L325R channels were co-transfected in a 1:1 ratio in HEK293 cells, the peak I_{Na} density was reduced to only 29.8 ± 6.2% of control. Surprisingly, a similar dominant-negative effect was also observed when L325R-*SCN5A* was coexpressed with the WT skeletal muscle sodium channel (WT-*SCN4A*). Moreover, when L325R channels were expressed in neonatal rat ventricular myocytes (NRVM), the peak I_{Na} was also reduced by about 80% (peak current density at −10 mV=-32.5+14.9pA/pF), compared to non-transfected NRVMs (peak current density at −10 mV=-182.7+36.3pA/pF). As expected, overexpression of WT-*SCN5A* increased the peak I_{Na} (peak current density at −10 mV=-234.6+32.2pA/pF). Interestingly, L325R did not interfere with the proper function of I_{K1} in NRVM, suggesting a specific dominant negative effect of L325R channels on sodium channels. In conclusion, the dominant negative effect observed in some BrS mutations suggests an interaction of sodium channel α-subunits which appears to occur even across different sodium channel isoforms.Our findings demonstrate that the L325R allele exerts a dominant negative effect on WT channels not only in heterologous expression systems but also in native cardiomyocytes, which would explain the BrS phenotype seen in patients carrying this mutation.

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Trauma-Induced Nav Leak and Dysexcitability in Axonal Membranes: Simulating the Consequences of Mechanically-Induced Left-Shift of Transient Nav1.6 Current

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Several experimental findings motivate our simulations: 1) immediately after mechanical trauma, CNS nodes of Ranvier exhibit axolemmal blebs; 2) upon mechanically-induced bleb formation, the operation of recombinant Nav1.6 channels (= node of Ranvier Nav isoform) becomes irreversibly shifted towards hyperpolarized potentials; 3) mechanically-traumatized axons (and Nav1.6-expressing HEK cells) exhibit TTX-sensitive Na^+ leak; 4) mechanical trauma to axons causes diverse excitability problems. It has been assumed (without direct evidence) that trauma increases persistent Nav current but we wondered if various trauma-induced pathological states (e.g. hyperexcitability and spontaneous discharge as seen in neuropathic pain, conduction block as seen in a knock-out punch, excitotoxic rise in axoplasmic Ca^{++} as seen in diffuse axonal injury) could be attributed to left-shifted operation of transient Nav current. We therefore modeled excitability, ion homeostasis and action potential propagation in myelinated axons (one or several nodes of Ranvier) with Nav channels of varying fractions of the axolemma left-shifted to various extents. Left-shift of activation and availability of Nav channels shifts window current (i.e. steady-state current through transient channels) leftward. Even if persistent current left-shifted as well, window current, we found, would be the critical source of Nav leak and could trigger degenerative excitotoxic cascades. Further, we show that, depending on the extent of left-shift and the fraction of affected axolemma, displaced window current predicts conduction block as well as assorted forms of hyperexcitability (including excitatory subthreshold oscillations, spontaneous ectopic excitation, reduced threshold for stimulated firing). In voltage clamp (as opposed to action potential) simulations, we demonstrate how various protocols could be used to test whether left-shifted Nav current in a traumatized membrane patch was an all-or-none or graded process.

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Patch Trauma-Induced Kinetic Changes in Nav1.4 Channels (Oocyte Patches): Gating in Both a Nav1.4-Nav1.5 Chimera Insusceptible to β1 Modulation and a Nav1.4 Mutant Incapable of Binding G-Ankyrin is Irreversibly Modulated by Membrane Stretch

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Oocytes lack endogenous Nav-modulating β subunits. Nav1.4α (unlike Nav1.5α) pore subunits expressed in oocytes exhibit anomalously slow, right-shifted voltage-dependent gating but co-expression with β1 subunits results in normal (i.e. more rapid, more left-shifted) Nav1.4 kinetics. For unknown reasons, membrane stretch due to pipette aspiration in cell-attached oocyte patches has the same effect on Nav1.4 α kinetics as co-expression of α with β1, namely, acceleration/left-shift of the activation and availability processes. This also occurs with Nav1.6 (Wang et al 2009 Am J Physiol 297:C823) where the nature of the kinetic changes in Nav1.6 suggests trauma-induced changes in bilayer mechanics as a plausible explanation. However, mechanical disruption between Navα and various protein ''targets'' might also be the explanation. Makita et al 1996 (J Neurosci 16:7117) generated a Nav1.4-Nav1.5 chimera in which the re-entrant S5-S6 ''pore loops'' of Nav1.4 domains 1 and 4 were substituted with the corresponding Nav1.5 pore loops. This rendered the chimera incapable of modulation by β1 subunits. Here we show that slow component activity in this chimera was irreversibly accelerated and left-shifted by membrane stretch. We also tested a rat Nav1.4α mutant in which the cytoplasmic loop 2 amino acids responsible for binding to G-ankyrin have been deleted. In this Nav1.4α mutant too, patch stretch irreversibly accelerated/left-shifted the anomalously slow activity of the channel. These findings rule out the possibility that the extracellular β1 interacting domains of Nav1.4α or the intracellular G-ankyrin interacting domain of Nav1.4α are required for the irreversible membrane trauma-induced acceleration/left-shift of this channel.

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Mitochondrial Reactive Oxygen Species Regulate the Cardiac Na^+ Channel

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Background: Pyridine nucleotides regulate the cardiac Na^+ current (I_{Na}) through PKC activation and reactive oxygen species (ROS), which is inhibited by NAD^+ through PKA activation. Here, we investigated the source of ROS induced by elevated NADH and how it affected the cardiac Na^+ channel ($Na_v1.5$).
Methods: HEK cells stably expressing $Na_v1.5$ and rat neonatal ventricular myocytes were utilized. Effects on I_{Na} were assessed by whole-cell patch clamp recording. Also monitored were ROS generation, mitochondrial membrane potential ($\Delta\Psi_m$), and myocytes action potential (AP).
Results: Decrease of I_{Na} (52 ± 9%; $P<0.01$) induced by NADH (100 μM) in HEK cells was reversed by mitoTEMPO, rotenone, malonate, DIDS, PK11195 and 4'-chlorodiazepam. Antimycin A (20 μM) also decreased I_{Na} (51 ± 4%, $P<0.01$), which was blocked by NAD^+, forskolin, or rotenone. Inhibitors for complex IV, nitric oxide synthase, NADPH oxidases, xanthine oxidases, mitochondrial permeability transition pore, and mitochondrial ATP-sensitive K^+ channel did not alter NADH effect on I_{Na}. Analogous results were observed in myocytes. Rotenone, mitoTEMPO, and 4'-chlorodiazepam also blocked the effect of a mutant A280V glycerol-3-phosphate dehydrogenase 1-like protein on reducing I_{Na}, indicating a mitochondrial role in Brugada syndrome. Fluorescent microscopy revealed that elevated NADH led to mitochondrial ROS generation but did not affect the mitochondrial $\Delta\Psi_m$. NADH treatment did not affect the resting potential and the AP duration of myocytes. Nevertheless, a decrease of the maximum upstroke velocity of action potential (68 ± 12%, $P<0.05$) was observed.
Conclusions: Altering the oxidized to reduced NAD(H) balance can activate mitochondrial ROS production from complex I/III and release from the mitochondrial inner membrane anion channel leading to reduced I_{Na}. This signaling cascade may help explain the link between altered metabolism, conduction block, and arrhythmic risk.